

## EXAMPLE 30

Example 30 demonstrates the effect of the PEG-ceramide concentration on the encapsulation efficiency by the dialysis method with 7.5% DODAC and DOPE. The non entrapped DNA in the various formulations with different PEG-C14 concentrations was separated by DEAE Sepharose CL6B chromatography. DNA and lipid recovered are shown as a function of % PEG-C14. Best entrapment was obtained with 10 mol % PEG-C14. FIG. 46. However, a more recent experiment showed optimum entrapment in the range of 10 to 15 mol % (data not shown).

## VII. Conclusion

As discussed above, the present invention comprises novel lipid-nucleic acid complexes and methods of making them. In a number of embodiments, hydrophobic DNA intermediates can be isolated and the DNA exists in a non-condensed form as measured by dye binding and DNase I sensitivity. These complexes can be used in the preparation of other lipid-nucleic acid particles.

In further embodiments, the invention provides methods for preparing serum-stable nucleic acid-lipid particles which are useful for the transfection of cells, both in vitro and in vivo.

The methods described for the preparation and uses of the various nucleic acid particles can be used with essentially any nucleic acid which can exist in a lipophilic state when complexed with an appropriate cationic lipid. Examples of some constructs include those encoding adenosine deaminase, the low density lipoprotein receptor for familial hypercholesterolemia, the CFTR gene for cystic fibrosis, galactocerebrosidase for Gaucher's disease, and dystrophin or utrophin into muscle cells for Duchenne's muscular dystrophy.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification for all purposes to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be-obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A method of introducing a nucleic acid into a cell, said method comprising contacting said cell with a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a nucleic acid, wherein said nucleic acid in said nucleic acid-lipid particle is resistant in aqueous solution to degradation with a nuclease.

2. The method of claim 1, wherein said particle is substantially non-toxic.

3. The method of claim 1, wherein said particle has a median diameter of less than about 150 nm.

4. The method of claim 1, wherein said cationic lipid is a member selected from the group consisting of N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleoyloxypropylamine (DODMA), and a mixture of two or more of the above.

5. The method of claim 1, wherein said particle further comprises a non-cationic lipid.

6. The method of claim 5, wherein said non-cationic lipid is selected from the group consisting of DOPE, POPC and EPC.

7. The method of claim 1, wherein said conjugated lipid is a PEG-lipid.

8. The method of claim 7, wherein said PEG-lipid comprises from 1% to about 15% of the total lipid present in said particle.

9. The method of claim 7, wherein said PEG-lipid is PEG-ceramide.

10. The method of claim 9, wherein the ceramide of said PEG-ceramide comprises a fatty acid group having 8 carbon atoms.

11. The method of claim 9, wherein the ceramide of said PEG-ceramide comprises a fatty acid group having 14 carbon atoms.

12. The method of claim 9, wherein the ceramide of said PEG-ceramide comprises a fatty acid group having 20 carbon atoms.

13. The method of claim 7, wherein said PEG-lipid is PEG-phosphatidylethanolamine.

14. The method of claim 1, wherein the nucleic acid:lipid ratio within said particle is at least 5 mg nucleic acid per mmol lipid.

15. The method of claim 1, wherein the nucleic acid:lipid ratio within said particle is at least 20 mg nucleic acid per mmol lipid.

16. The method of claim 1, wherein the nucleic acid:lipid ratio within said particle is at least 40 mg nucleic acid per mmol lipid.

17. The method of claim 1, wherein said nucleic acid is DNA.

18. The method of claim 1, wherein said nucleic acid is a plasmid.

19. The method of claim 1, wherein said nucleic acid is an antisense oligonucleotide.

20. The method of claim 1, wherein said nucleic acid is a ribozyme.

21. The method of claim 1, wherein said cationic lipid comprises 50% or less of the lipid present in said particle.

22. The method of claim 1, wherein said cationic lipid comprises from an amount greater than 0% to about 20% of the lipid present in said nucleic acid-lipid particle.

23. The method of claim 1, wherein the nucleic acid component of said particle is substantially not degraded after exposure of said particle to a nuclease at 37° C. for 20 minutes.

24. The method of claim 1, wherein the nucleic acid component of said particle is substantially not degraded after incubation of said particle in serum at 37° C. for 30 minutes.

25. The method of claim 1, wherein more than 10% of a plurality of such particles are present in plasma one hour after intravenous administration.

26. The method of claim 1, wherein said cell is present inside of a mammal, and wherein said transformation of said cell by said particle at a site distal to the site of administration is detectable for at least four days after intravenous injection.

27. The method of claim 1, wherein said cell is present inside of a mammal, and wherein said nucleic acid-lipid particle is administered parenterally to said mammal.

28. The method of claim 27, wherein said particle is administered to said mammal by intravenous injection.

29. The method of claim 27, wherein said particle is administered to said mammal by intraperitoneal delivery.